that are responsible for particular pathological conditions. Thus, although many methods for detection of both known and unknown mutations have been developed (e.g., see Cotton, 1993), our growing knowledge of human and other genomes makes it increasingly important to develop new, better, and faster methods for characterizing nucleic acids. Besides diagnostic uses, improved methods for rapidly characterizing nucleic acids will also be useful in many other areas, including human forensics, paternity testing, animal and plant breeding, tissue typing, screening for smuggling of endangered species, and biological research.

One of the most informative ways to characterize a DNA molecule is to determine its nucleotide sequence. The most commonly used method for sequencing DNA at this time (Sanger, et al., 1977) uses a DNA polymerase to produce differently sized fragments depending on the positions (sequence) of the four bases (A = Adenine; C = Cytidine; G = Guanine; and T = Thymine) within the DNA to be sequenced. In this method, the DNA to be sequenced is used as a template for *in vitro* DNA synthesis. RNA may also be used as a template if a polymerase with RNA-directed DNA polymerase (i.e., reverse transcriptase) activity is used. In addition to all four of the deoxynucleotides (dATP, dCTP, dGTP and dTTP), a 2',3'-dideoxynucleotide is also included in each *in vitro* DNA synthesis reaction at a concentration that will result in random substitution of a small percentage of a normal nucleotide by the corresponding dideoxynucleotide. Thus, each DNA synthesis reaction yields a mixture of DNA fragments of different lengths corresponding to chain termination wherever the dideoxynucleotide was incorporated in place of the normal deoxynucleotide.

The DNA fragments are labelled, either radioactively or non-radioactively, by one of several methods known in the art and the label(s) may be incorporated into the DNA by extension of a labelled primer, or by incorporation of a labelled deoxy- or dideoxy- nucleotide. By carrying out DNA synthesis reactions for each of the four dideoxynucleotides (ddATP, ddCTP, ddGTP or ddTTP), then separating the products of each reaction in adjacent lanes of a denaturing polyacrylamide gel or in the same lane of a gel if different distinguishable labels are used for each reaction, and detecting those products by one of several methods, the sequence of the DNA template can be read directly. Radioactively-labelled products may be read by analyzing an exposed X-ray film. Alternatively, other methods commonly known in the art for detecting DNA molecules labelled with fluorescent, chemiluminescent or other non-radioactive moieties may be used.

Because 2',3'-dideoxynucleotides (ddNTPs), including even ddNTPs with modified nucleic acid bases, can be used as substrates for many DNA polymerases, Sanger's dideoxy-sequencing method is widely used. Recently, Tabor and Richardson (EP application 942034331, 1994) reported that mutations at specific sites in many DNA polymerases improved the ability of these mutant enzymes to accept ddNTPs as substrates, thereby leading to improved DNA polymerases for DNA sequencing using the Sanger method.

Nucleic acid sequencing provides the highest degree of certainty as to the identity of a particular nucleic acid. Also, nucleic acid sequencing permits one to detect mutations in a gene even if the site of the mutation is unknown. Sequencing data may even provide enough information to permit an estimation of the clinical

significance of a particular mutation or of a variation in the sequence.

Cycle sequencing is a variation of Sanger sequencing that achieves a linear amplification of the sequencing signal by using a thermostable DNA polymerase and repeating chain terminating DNA synthesis during each of multiple rounds of denaturation of a template DNA (e.g., at 95°C), annealing of a single primer oligonucleotide (e.g., at 55°C), and extension of the primer (e.g., at 70°C).

Other methods for sequencing nucleic acids are also known besides the Sanger method. For example, Barnes described a method for sequencing DNA by partial ribo-substitution (Barnes, W.M., 1977). In this method, a pre-labelled primer was extended *in vitro* on a template DNA to be sequenced in each of four reactions containing a wild-type DNA polymerase in the presence of Mn2+, all four canonical 2'-deoxyribonucleoside triphosphates, and one of four ribonucleoside triphosphates under deoxy-/ribo-nucleotide ratios and conditions that result in about 2% ribonucleotide substitution at each position. After alkali treatment to cleave the synthetic DNA at the positions of partial ribosubstitution, the sequence was determined by analyzing the fragments resulting from each reaction following electrophoresis on a denaturing polyacrylamide gel.

Although most methods for sequencing nucleic acids employ DNA polymerases, some work has also been reported on the use of T7 RNAP and SP6 RNAP for transcription sequencing of DNA templates beginning at the respective T7 or SP6 promoter sequence using 3'-deoxyribonucleoside-5'-triphosphates (Axelrod, V.D., and Kramer, F.R., 1985), and Q-Beta replicase for sequencing single-stranded RNA